SECTION II:
Origins and Arrival of Africans in Colonial New York

CHAPTER 5

Origins of the New York African Burial Ground Population: Biological Evidence of Geographical and Macroethnic Affiliations Using Craniometrics, Dental Morphology, and Preliminary Genetic Analyses

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Introduction and Theoretical Perspectives

Origins are central to understanding the past and present identity of a people. Origin studies provide, under optimal conditions, a context for all other assessments, such as archaeological, biomedical, and nutritional evaluations. Characterizing the phenotypic status and determining the origins of the eighteenth century New York African population and those individuals interred in the New York African Burial Ground (NYABG) were among the project's major goals. Towards that end, our craniometric, dental morphology, and genetic teams collaborated extensively with project historians and archaeologists to develop a biocultural, interdisciplinary research strategy for a historically and ethnographically informed interpretation of the ancestral origins of the people disinterred from the NYABG. Our research strategy addressed the inquiries of the descendant African-American community with the professional scientific rigor demanded by our disciplines.

At this point in our investigations, the craniometric and dental morphology data are the most complete and well-developed lines of evidence for establishing relationships between the African Burial Ground (ABG) individuals and other world groups. Craniometric evaluations for the purpose of ancestral origin determinations have been

applied to anthropological populations for a very long time and have a rich history. Classically such studies were focused on typological racial assessments. This was not the case in the studies by Froment and Keita and Shujaa that quantify craniometric diversity and then compare this variability with a broad range of historical and modern African and non-African groups. Dental morphology studies are a well-established basis for assessing presumable genetic relationships between skeletal populations. This chapter contains a dental morphology study by Mayes and Mack that addresses the biological diversity in dentition observed among the NYABG individuals and the historical population affiliations associated with this variability. Both the craniometric and dental morphology studies confirm the African regional backgrounds of the New York population and probe the current limits for establishing greater ethnic specificity using such traditional methodology and comparative statistics.

Molecular genetic assessment, our third approach to determine ancestral origins, is a rapidly emerging and extremely precise set of techniques to match individuals with specific geographical regional groups, often with a high degree of reliability. Preliminary genetic studies by George and Kittles suggest that, based upon DNA analysis, much of the genetic diversity characterizing a subsample of NYABG individuals is decidedly West and Central African in origin. In a few cases, these preliminary results could localize specific individuals to precise geographical regions of West and Central Africa and suggest macroethnic affiliations. These preliminary studies, with a subset of the NYABG individuals, clearly point to the feasibility and utility of continued research effort in this area. Jackson‘s initial work provides a roadmap for these future studies.

Database limitations, research strategies, and historical and evolutionary contexts

Despite this focus, all four bioanthropological lines of evidence, dental morphology, non-metric phenotypic traits, craniometrics, and molecular genetics encountered problems in the comparative analysis of the data. The most significant problem encountered was the dearth of appropriate, non-racialized studies of African, Native American, and European population biology diversity for comparative assessments with the individuals of the ABG. Of particular note is the continued paucity of data on intra-African diversity. For example, substantive non-metric trait studies that include African populations are not available in the published literature. This important limitation in the database is being partially addressed at a number of levels, including the development in 2002 of the first human DNA bank in Africa by Jackson and her colleagues (Mbah 2003). The aim of this project, as a direct response to the needs of the NYABG analyses, is to have a full representation of and public access to continental African molecular genetic diversity that can be linked to geographical region, ecological setting, national identity, and ethnicity. Similar efforts are underway to characterize the genetic diversity among East Coast Native American groups (Smith, personal communication 2003).

Another limitation of our sample for broader extrapolations is that the Africans and African Americans enslaved in New York represent a distinct minority of North American captives. In 1790, there were nearly half a million Africans and African Americans enslaved in the United States. Of these 491,157 individuals, only 21,193 (approximately 4.3 percent of all enslaved persons) resided in New York State. The majority of these individuals were in New York City. Therefore, the representativeness

of this subset of North America's enslaved population is likely problematic. The origins of the NYABG individuals may not reflect the origins of enslaved Africans elsewhere in the Americas.

A third limitation in extrapolating our studies is the size of the subset of retrieved individuals from the NYABG. The approximately 400 individuals retrieved from an estimated 15,000 interred represent less than 3 percent of the estimated total number of buried individuals. The representativeness of this subset for all of New York City's enslaved persons is likely problematic. The retrieved individuals come from a limited area of the actual Burial Ground and may represent a clustering of genetically related and/or phenotypically affiliated individuals. Indeed, a preliminary analysis of mtDNA based interrelationship in a small group of interred individuals suggests close maternal affiliations between particular burials. Additional genetic tests based on Y-chromosome haplotypes and autosomal genes are warranted to further illuminate the genetic interrelationship of the individuals recovered from the NYABG along with molecular sex determinations of all individuals.

Finally, for a number of historical and evolutionary reasons, it is very difficult to reconstruct the exact modern or historical African ethnic group(s) to which a specific NYABG individual belongs, since New World Africans (including African Americans) are highly heterogeneous and represent an amalgamation of genes from diverse African ethnic groups in addition to highly variable genetic contributions from non-Africans (primarily Europeans and Native Americans). Many of the European slaving vessels would pick up shipments of captive Africans from various points on the coasts of Africa. In gathering captives in this manner, the vast majority of shipments contained a rainbow

of ethnicities that eventually found their way to the docks, households, and plantation work sites of the Americas. This created a plethora of African ethnicities with maximal opportunities for gene flow between individuals that had, on the continent of Africa, remained distinct. On the small island of Dominica alone, there were captives from diverse areas in Africa such as Old and New Calabar, Gambia, Cape Mount, Angola, Bonny, Cameroon, and Anamaboo (Carrington, personal communication, 2002). These names only refer to the regions from which the Africans were acquired for shipment, not their specific ethnic groups. A similar scenario is anticipated for New Amsterdam/New York City, thus complicating the efforts to link specific Burial Ground individuals to particular African ethnic groups. As the specificity of the African databases improves, we should be able to detail regional and macroethnic levels of genetic nuance. Indeed, our preliminary molecular genetic studies have already allowed this level of sophisticated assessment.

The Atlantic trade in 10-50 million enslaved Africans and the interactions of the survivors and their descendants in the Americas provide the ancestral foundations for the individuals of the NYABG. Both the geographical extensiveness of the trade in enslaved Africans, often deep into the interior of the continent, and the diversity of their interactions with each other and with non-Africans (Europeans and Native Americans) warrant that a broad array of regional groups be included for origin reconstruction studies. This is reflected in the research strategies implemented for the craniometric, dental morphology, and molecular genetic components of our study; comparisons were made with available African, Native American, and European groups. However, the dominant African origins of NYABG individuals became evident early in our analyses.

Once the primacy of African origins was obvious from craniometric, dental, and molecular genetic data, we sought to further refine our studies to identify where in Africa these individuals may have had their strongest ancestral ties.

The historical record indicates ten major geographical regions as sites from which enslaved Africans were likely exported to New Amsterdam/New York during the time frame of the NYABG (Figure 5.1 and 5.2). Additionally, the origin, number, initial entry points and subsequent re-exportation routes of enslaved Africans to the Americas (e.g., Caribbean trade to New York) have been identified (History, Report Chapters 2, 6, and 7). These historical data help us better understand the potential for genetic and phenotypic variations among the NYABG individuals, based upon the likelihood of gene-flow among previously diverse Africans, gene-flow with non-Africans, possibilities of genetic drift and bottleneck effects, and various types of selection at particular points in the historical record. The historical information also suggests the ethnic and regional identities of potential reference ancestral groups (e.g., which specific groups of Native Americans may have biologically interacted with the Africans of New York) as well as the characteristics of the databanks (e.g., distinctive morphological traits) necessary to ascertain the origins of New York Africans interred in the Burial Ground.

The larger context for reconstructing the origins of the NYABG individuals is within the current paradigm of modern human origins. The available molecular and skeletal information on recent human evolution favors a recent African origin of modern humans who spread out of Africa approximately 100,000 to 200,000 years ago (Ayala and Escalante 1996). In this context then, non-African diversity represents a subset of African heterogeneity, complicating somewhat our search for continental and population-

specific phenotypic and genotypic markers. However, for all of the useful polymorphic traits studied, African levels of diversity have exceeded those observed in non-Africans, and much of the African diversity appears to be clustered geographically and/or ethnically. Theoretically, this implies that genetic and phenotypic assessments of the NYABG individuals should be able to identify whether they are of predominantly African or non-African origin and, if they are African, which regions of the continent they share ancestral affinities. Furthermore, existing craniometric, dental morphology, and molecular genetic variation allows us to characterize these individuals in relation to themselves and to address questions of kinship within the group.

Figure 5.1: Major African exit points for enslaved individuals bound for New York in the 17th and 18th century. Additional exit points (not shown) include Mozambique and western Madagascar as well as the Atlantic islands off of West Africa (e.g. Cape Verde).

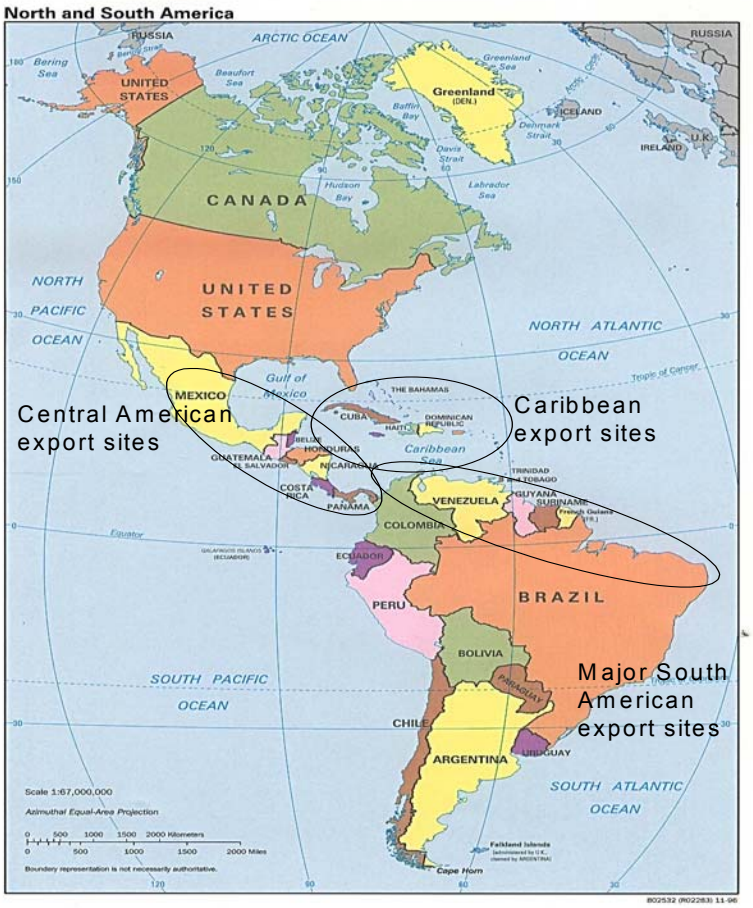

Figure 5.2: Exit regions for enslaved Africans bound for New York, Central and South American and the Caribbean in the seventeenth and eighteenth century.

RESEARCH QUESTIONS

The four major questions to be addressed in using genetics and phenotype to reconstruct the ancestral origins of the NYABG population are:

1. Is it possible to differentiate between continental groups (Africans, Europeans, Native Americans as a subset of Asians) at the genetic and/or phenotypic levels?
2. Is it possible to differentiate genetically and/or phenotypically in the ABG sample, among the ancestral Africans, ancestral Europeans, and ancestral Native Americans

coming from various historically relevant geographical areas and germane ethnic groups within a specific continent?

3. Is it possible to differentiate sex-linked differences in ancestral origins and biological affinity among those interred in the NYABG?
4. Most importantly, is it possible to differentiate among the Africans, who most likely contributed disproportionately to the ancestral backgrounds of those interred in the NYABG, from various regions of Africa and between different macroethnic groups of Africa?

In other words, we are especially interested in the complex relationships of this sample population to each other and to the larger world. These distinct but complementary levels of assessment are critical for ascertaining the origins of those interred in the NYABG. The New York African Burial Ground Project (NYABGP) research team has begun to notably contribute to the existing databases.

RESEARCH BACKGROUND SYNOPSIS

The importance of integrating phenotypic and genotypic variation in our assessments of biological lineage and ancestral origins is evident from the wealth of such studies in the published literature. There is considerable variation between and within populations (e.g., with regard to such traits as tooth size, congenitally missing teeth, crown morphology, mtDNA haplogroups, etc.). These differences are a reflection of the ongoing process of evolution and can be used to accurately reconstruct ancestral origins in specific populations when contextualized by an appropriate understanding of history and the environment.

Craniometric Assessments

Assessments of craniometric variation from Africa, Europe, and Asia basically support the dominant African-centered genetic and archaeological models of human origins and microevolution (Relethford and Jorde 1999). The average heterozygosity is significantly higher among Africans indigenous to the sub-Saharan areas of the continent than among non-Africans. An early study (Relethford and Harpending 1994) of worldwide variation in within-group phenotypic variation applied to a large set of craniometric data representing major Old World geographic regions involved 57 measurements for 1,159 cases in four regions: Europe, Sub-Saharan Africa, Australasia, and the Far East. Relethford and Harpending predicted a linear relationship between variation within populations (the average within-group variance) and variation between populations (the genetic distance of populations to pooled phenotypic means). If this prediction continues to hold true, craniometric data should also facilitate our hypothesis testing of ancestral origins of individuals retrieved from the NYABG.

Dental Trait Variants

The study of teeth has historically been an informative means of demonstrating patterns of human dispersals (Shields 1999). The multivariate analysis of worldwide dental phenotype microevolution suggests that world patterns are also broadly in accord with the dominant interpretation of genetic, archaeological, and other dental data. Like these data, dental morphology suggests an African (i.e., San, Western Africans, and Bantu) origin and subsequent dispersal for extant humanity. According to a prevailing interpretation of dental trait variation, the first modern human African emigrants not to become extinct were Southeast Asian Negritos. All Eurasians then emerged and

expanded through a series of extinct antecedent populations branching from the short lineage extending from Negritos to Australian aborigines. Proto Europeans were the first group to fission from this lineage. Under this dental morphology-generated hypothesis of modern human origins and subsequent differentiation, the next groups to have emerged were antecedent Southeast Asians, from which present Southeast Asians and then antecedent east Central Asians then diverged. Independently, people from the region of Mongolia and all Native Americans arose as daughter populations from antecedent East Central Asians (Shields 1999). Given this scenario, we should be able to find dental variants that distinguish between various continental groups of contemporary humans as well as high dental morphology diversity within Africa.

Fortunately, the study of European genetic diversity has been quite extensive, and the characterizations of the relevant groups for our intracontinental comparisons are more advanced. Of greatest relevance to our testing of hypothesis two is evidence of genetic and phenotypic diversity among the Dutch, Spaniards, Portuguese, English, Scots, Irish, French, Danes, Germans and others since these groups were the most active in the transatlantic trade in enslaved Africans and/or maintained the greatest potential (due to proximity) for contributing to the gene pool of the New York population.

Molecular genetics

Applied genetics is of increasing relevance in our efforts to reconstruct the origins of long deceased populations. New methods, new techniques, and the increased ease at which sophisticated assessments can be made have provided new ways of knowing the long buried histories of individuals and, by extension, their groups. In a recent article by senior geneticists Cavalli-Sforza and Feldman (2002), they note that the past decade of

advances in molecular genetic technology has heralded a new era for all evolutionary studies, but especially the science of human evolution. Data on various kinds of DNA variation in human populations are rapidly accumulating, particularly markers from mitochondrial DNA (mtDNA) and the Y chromosome. The evolution of the human mitochondrial genome is characterized by the emergence of geographically distinct lineages or haplogroups. Significant differences between the three African, nine European, and seven Asian (including Native American) haplogroups make it now possible to confirm or reject an African genetic origin from studies using mtDNA. Indeed, the analysis of nucleotide sequences of the D-loop of mtDNA derived from the aDNA of a small sample of NYABG individuals has been very informative in confirming the continental origin (Africa) and in its subsequent elaboration of the geographical region, within-continent identities of nearly 50 NYABG individuals.

Methods, Data, and Results

Craniometrics

The scope of the present study is not about human variation in general, and therefore does not include populations from all around the world; the issue is to assess the origins of the people buried in the cemetery as they represent the New York African community of the time. These origins combine three roots:

1. The geographical origin in Africa (according to the historical analysis the primary sources of the enslaved population was from Central and Western Africa, with a minor Madagascan and Southern African components)
2. Some admixture with the European colonists in America and traders in Africa
3. Some admixture with Native Americans

To undertake such a comparative study, it was necessary to first build a reference collection of Europe, African, and Native American populations, ideally of the same period (circa 1650 to 1769). Two decisions were made: consider only adults, and limit comparisons to skull measurements. The first decision was based on the fact that there are virtually no studies on subadult skeletons, due to the under representation of subadults in cemetery populations (due to preservation and/or cultural processes), the disarticulated nature of subadult crania, and the difficulty in determining sex until late adolescence (unless performing DNA testing).

The second decision, to study only crania, was based on the status of this type of research and not the potential utility of the post-cranial skeleton for assessing ecological variation. Unfortunately, most skeletal biological research concerning anatomical variation focus on stature, race, and sex assessments; while the primary research on anatomical variation has been developed within human adaptability research on living populations. These data have not been correlated with skeletal analyses. Therefore, the cranial element of the human skeleton remains the most studied element for comparative analysis and is still highly racialized.

A literature review for intercontinental and intracontinental crania variation was undertaken. The literature review revealed that there was a paucity of individual level data, and sample variation reported primarily by means and standard deviations only. In addition, limited standardized measurements (usually 6 to 15) were published, thus producing a loss of biologically relevant information. No reference to any "racial" definition was made as the multivariate analysis does not require it and the scatter plots speak for themselves to express the resemblance between individuals, without the use of

closed biological categories. Table 5.1 identifies the populations used in this study and the sources they came from.

Statistical Analysis

A stepwise discriminant function analysis was undertaken using the statistical software package, "Statistical Package for Social Sciences (SPSS)." The purpose of the analysis was to classify a series of unknown origin objects, the NYABG skulls, into groups defined on a geographical basis, by using simultaneously multiple variables (cranial measurements). A preliminary, univariate statistical analysis verified that the variables studied display a normal distribution. Then canonical discriminant functions are generated, each function expressing a part of the total variance and displaying, more or less, an important correlation with some of the discriminating variables. Distances between individuals are calculated and crania are plotted in a hyperspace of the same dimension as the number of variables considered. The Mahalanobis metric, a generalized Euclidean metric, is employed to measure the distance between two points in this hyperspace because it adequately accounts for correlated variables.

Associated with each group in a sample is a point called the group centroid, which represents the means for all variables in the hyperspace defined by variables in the model. A case is said to belong to a group if the Mahalanobis distance of the case from the group's centroid is smaller than the Mahalanobis distance from any other group's centroid.

Table 5.1: Population Sources for Craniometric Analysis by Froment

# Indiv.	Population	Reference
59	**NYABG**	Present study
	EUROPE	
271	City of Lyon (France)	Buyle-Bodin 1982
785	Valais (Switzerland)	Pittard 1911
131	City of Firenze (Italy)	Florence Museum collection
110	Norse (Norway)	Howells 1989
264	Grodek, Poland, 13-17th century	Belniak et al., 1961
	WEST AFRICA	
114	Ashanti (and other Ghanaians)	Shrubsall 1899a
71	Ibo and Calabar area (Nigeria)	*in* Ribot 2002
96	Senegal (Seerer and Iron Age)	*in* Ribot 2002
127	Dogon (Mali)	Howells 1989
134	Tellem (early Mali)	
22	Togo & Benin	*in* Ribot 2002
	CENTRAL AFRICA	
133	Cameroon (mainly Grassfields)	Drontschilow 1913
155	BaSuku D.R.Congo	*in* Ribot 2002
68	Various D.R.Congo (Zande, Kongo)	*in* Ribot 2002
82	Tetela, D.R.C.	Benington 1912
141	Bantu from Gabon	Benington 1912
	SOUTH AFRICA	
125	Zulu	Howells 1989, Shrubsall 1899b
27	Nguni	*in* Ribot 2002
48	Xhosa	*in* Ribot 2002
19	Various Bantu South Africa	*in* Ribot 2002
36	Ovambo	Hrdlička 1928b
	EAST AFRICA (just for comparison)	
30	Dschagga Kilimandjaro	Wide 1896
29	Teita (Kenya)	Kitson 1931
	NATIVE AMERICANS	
111	Algonquin (mainly Virginia)	Hrdlička 1927b
53	Connecticut, Delaware, Maine	Hrdlička 1927b
24	Huron	Hrdlička 1927b
41	Massachusetts	Hrdlička 1927b
115	Manhattan, Long Island, Rhode Island	Hrdlička 1927b
120	Central California	Breschini & Haversat 1980
89	Labrador "Indians"	Steward 1939b
43	**Guadeloupe** slave cemetery (French West Indies)	Courtaud *personal communication* 2002

NB: the study by Ribot (2002) is a large compilation of available literature, where complete references can be found; many series have been measured by Ribot, or M. Lahr, or G. Thilmans; unpublished studies by Froment have also been included.

The number of variables included for analysis must be smaller than the size of the sample; this condition was met in this study. The number of individuals in each subsample (n= 20-28) was greater than the number of variables which varied from 5 to 12 based on the completeness of the crania. When a cranium is incomplete, the number of variables is reduced; and no missing measurement attempt was replaced by an estimate. An individual with a missing variable was excluded from the analysis, thus explaining the variation in sample size. Therefore, the strategy was to maximize the number of individuals without minimizing the number of variables for analysis.

Results

Although the New York sample is heterogeneous craniometrically, this analysis indicates that a majority of NYABG individuals can be considered African. Four individuals (n= 20 to 28 complete skulls) are closer to Europeans than to Africans, yet are within the overlapping ranges of both geographic populations (Figure 5.3). Since the greater proportion of West and Central African crania are clustering closely, they are not distinguishable. Most NYABG crania are clearly primarily Central and West African, although four to five individuals are within the range of South Africa.

When comparing NYABG with a sample from a Guadeloupe, French West Indies cemetery, that has only enslaved Africans, both NYABG and Guadeloupe cluster with Africans (Figure 5.4). The Native American component in the NYABG sample could not be definitively confirmed; in fact only one individual was close to a Native American and both of these individuals were in the overlapping range with Europeans (Figure 5.4).

In any world population, there is considerable individual variation. In a sample of heterogeneous origin, such as the NYABG sample, variation is even greater. Yet, this

analysis demonstrates that those individuals interred in the burial ground were of African origin; what this analysis can not do is identify the specific geographic areas narrowly, nor can it identify specific African ethnic groups. Based on adaptation and ecological theory and lines of evidence, this should be possible, but only substantive, relevant reference populations would provide a means to test hypotheses and undertake these analyses.

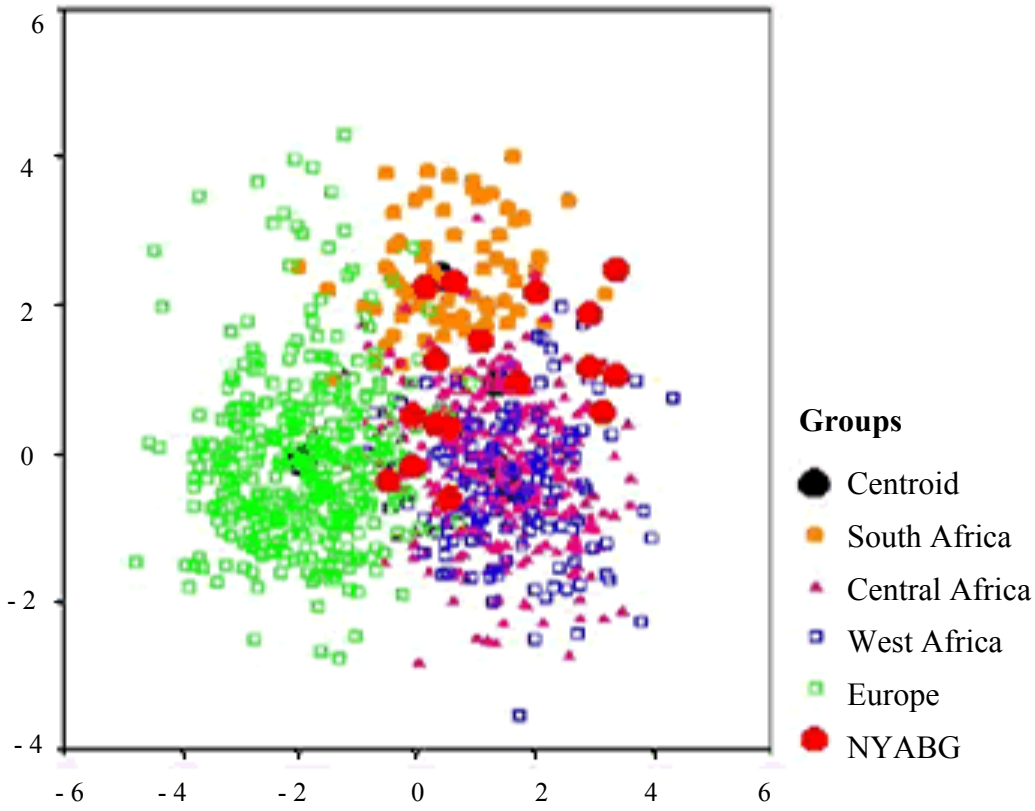

Figure 5.3: New York African Burial Ground Skull Shape Analysis (Mahalanobis Distance) NYBG population (red dots) compared to Southern and Northern Europe (green squares, n=357), West Africa (blue squares, n=115), Central Africa (pink triangles, n=342), South Africa (brown dots, n=59). Centroids are in black.

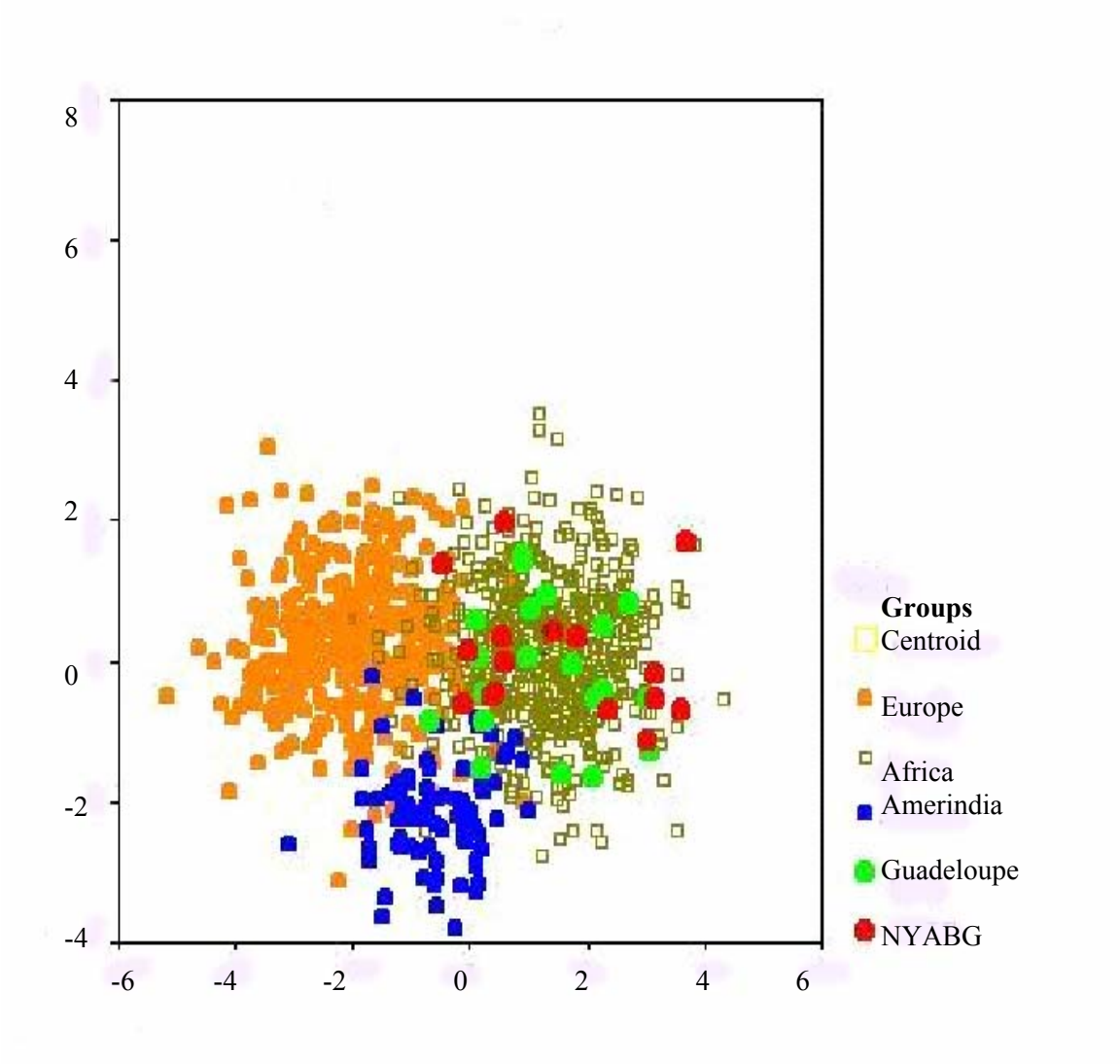

Figure 5.4: Scatter Plot of Craniometric Distance

New York African Burial Ground Population (Red Dots) Compared To All Europe (Orange Squares), All Africa (Olive Squares), Native Americans (Blue Squares), and a Xvii-Xix Century Cemetery from Guadeloupe, French West Indies (Green Dots).

In another craniometric analysis done by Keita and Shujaa, exclusively male crania were used to assess population affinities using the requirement of at least 10 measurements per skull because more of these were intact or sufficiently reconstructed (n=26 with at least 10 standard measurements) than female crania for the required sex-specific analysis. These crania are from burials distributed across the site. The comparative material is from Howells, (1973) study (Table 5.2), and measurements of crania from Gabon taken by Keita, and crania measured by Shujaa at the American Museum of Natural History (AMNH) for the ABGP researchers (Table 5.3).

Ten craniometric variables were taken: maximum breadth, biauricular breadth, basibregma height, maximum length, upper facial height, nasal breadth, nasal height, bizygomatic breadth, basion-prosthion length, and basi-nasion length. Using the Statistical Package for the Social Sciences, the ABG cranial series was analyzed with the others using canonical discriminant functions (Table 5.4). The centroid values place the New York crania nearest series from the Akan-speaking Ashanti (Asante) and Gold Coast series of the AMNH which form the modern nation of Ghana. Statistical proximity to populations that are historically unlikely to have had an opportunity to contribute genes to the New York population may exhibit morphological similarity that is unrelated to any close lineage affiliations. Thus Gold Coast individuals may be considered relevant candidates for a parental population to the NYABG, while others, such as the Tolai of New Guinea, are historically implausible.

The first two functions account for 54 percent of the variance, with eigenvalues of 2.03 and 1.05, respectively. The results of this study are consistent with known historical data. Many Africans were brought to English colonial America from what are now the

modern nations of Ghana, Angola, Democratic Republic of Congo, and the Senegambia Region [modern day Senegal and Gambia] (Curtin 1969; Inikori 1982; Gomez 1998).

Future research should include an analysis that only uses skeletal series from the regions of Africa that "contributed" most heavily in the seventeenth through eighteenth centuries to the Africans who were captured and enslaved in New York. The ABGP collected primary data on Ghanaian populations at the AMNH, which enabled a more diverse array of ethnic affiliations than was previously available from the published data on historical populations. Previous genetics and physical anthropological research has not focused on the question of African-American ethnic origins and thus has not over time produced a body of literature and appropriate comparative series for such analyses to be readily undertaken. Where African affinities or admixture have been studied, "racial" composites of diverse "sub-Saharan" or at best "West African" groups were constructed for analysis. The discussion of dental morphology below raises similar issues to these. There is a clear need to collect metric data on culturally-specific, historically relevant comparative populations in order to fully examine the range of NYABG origins.

In fact, much the same can be said of colonial English and Dutch populations. The closest Western European groups available in the Howells series (the most extensive series, generously provided to us by its author) are Scandinavian. The American Museum of Natural History collections (also graciously made available by Dr. Ian Tattersall) do not have a European series. Alternate collections that include the appropriate populations have been identified, and every effort will be made to include them in future studies. The current study, nonetheless, contributes substantially to the

search for origins of New York's earliest Africans by pushing the limits of current reference collections and showing the general craniometric affinities of the ABG sample. This study has demonstrated statistical relationships to specific ethnic populations to the extent available, although these statistical relationships need always to be measured in relation to historical plausibility. Furthermore, this examination has pointed to problems and their likely solutions if more robust ethnically-specific research on diasporic origins is to be conducted in the future.

Table 5.2: Howells Cranial Series

	Population Source		Population Source
1.	Norse (Norway)	16.	N. Japan
2.	Zalavar (Hungary)	17.	S. Japan
3.	Berg (Sweden)	18.	Hainan
4.	Teita (Kenya)	19.	Atayal
5.	Dogon (Mali)	20.	Philippines
6.	Zulu (South Africa)	21.	Guam
7.	Australia	22.	Egypt (Ancient, Late Period)
8.	Tasmania	23.	Bushman
9.	Tolai (New Guinea)	24.	Andaman Islands
10.	Mokapu (Hawaii)	25.	Ainu
11.	Easter Island	26.	Buriat (Siberia)
12.	Moriori	27.	'Eskimo'
13.	Ankara (Plains Indians)	28.	Anyang
14.	Santa Cruz	29.	S.Maori
15.	Peru	30.	N.Maori

Table 5.3: AMNH and Keita's Cranial Series

31.	Angola	36.	New York City
32.	Ashanti (Ghana)	37.	Staten Island
33.	Congo	38.	Gabon
34.	"Gold Coast"(Ghana)	39.	NYABG
35.	New York City		

Table 5.4: Centroid Values, Functions 1 and 2

Canonical discriminant functions evaluated at group means (group centroids)

Group	Function I	Function 2
1	.49289	-.81395
2	.66661	-.28289
3	2.00005	-1.33064
4	-1.83911	-.47633
5	-1.07939	-.80471
6	-1.56471	-.25812
7	-2.47642	-.62274
8	-1.31698	-.54909
9	-2.02165	.46116
10	.52011	1.54096
11	-1.39044	2.25476
12	1.09593	.72961
13	1.83141	.32525
14	.69772	-.77958
15	.76860	-.81823
16	.80924	.35667
17	.36571	.73132
18	.76538	.61597
19	.23183	.21099
20	.61612	.47115
21	.65600	1.76339
22	.24024	-5.6196
23	-1.62934	-2.49426
24	-1.8969	-.99949
25	-.21452	.18726
26	3.69605	-.63395
27	-.28503	1.58377
28	.61646	1.41405
29	1.02113	1.25171
30	-.26710	1.21620
31	-1.91646	.08740
32	-2.30512	.17624
33	-1.05963	-.37668
34	-2.02701	.17210
35	.23124	1.27807
36	1.81689	.18109
37	.15982	2.97094
38	-.82579	.50584
39	-1.50629	.52638

Dental Morphology

Analyzed in this section are dental trait frequencies and their intra and inter-population differences. Different populations appear to have similar ranges of dental cusp variation. As with other inherited traits, however, some distinct shapes of dental crowns (cusp patterns, for example) occur more often in particular regions, ethnic groups, and families than in others. This section examines an extensive range of dental variation in more than 200 individuals from the NYABG site. This part of the chapter explores the possible origins of the Africans in colonial New York City by comparing the frequencies of different dental shapes (morphology) found in the NYABG sample to those of historically relevant, potentially paternal populations in Africa, Europe, and North America. Using specific dental traits, several pertinent questions arose: how does the NYABG sample compare to other world populations? How does this sample compare to other African populations, based on the dental evidence? What is the relative degree of relationship within the population? Given the history of slavery on the African continent, can we determine the region of Africa in which this population originated, based on dental morphology?

Dental Comparison of New York African Burial Ground Individuals with Populations of the World

Variations in the degree and type of expression of dental morphology can be shown for different regional groups. These geographical and cultural patterns of frequency distribution are used to generate hypotheses about historical population relationships. This fact and the archaeological sturdiness of enamel make dentitions valuable repositories of information on migration. Scott and Turner (1997) have recently provided an in-depth and detailed review of discrete dental variation among modern and

recent human populations. This analysis incorporates data collected by many researchers during the past few decades. However, while extensive in scope, it should be noted that data for certain traits we have noted are not included for many of the populations presented by Scott and Turner.

In their analyses of biological distance, Scott and Turner used Nei's distance statistic and the hierarchical clustering algorithm known as the unweighted pair-group method using arithmetic averages (UPGMA) to produce trees or dendrograms. As with most multivariate clustering techniques, there is no direct way to evaluate significance or error. They did find, with their rather large samples, that they would get essentially the same dendrograms no matter what combination of a standard distance measure and clustering algorithm they employed (Scott and Turner 1997: 288). Turner's analysis is based on single-trait frequencies on a single tooth on one side of the dental arcade. This is true even for traits that potentially can be exhibited across dental fields, as well as bilateral occurrences. This technique is favorable for a more complete collection, which is taphonomically in good shape and which enables the researcher to control for large amounts of data. The dendrogram in Figure 5.5, below, shows the relationship between worldwide populations based on twenty-three crown traits (Scott and Turner 1997). It contains five clusters of world groups: Western Eurasia, Africa, Sunda-Pacific, Sahul-Pacific, and Sino-American. The African cluster is made up of two sub-groups, West Africa and South Africa. This larger world classification of Africa is more closely aligned with the Sunda-Pacific populations than with the Western Eurasia populations. In Scott and Turner's analysis, North Africa finds itself clustered with Western Eurasia. The same twenty-three dental traits used by Scott and Turner were compared to the data

set from the NYABG sample (Table 5.5). The SPSS average lineage (UPGMA) with Euclidean distance as the distance statistic was used to produce our dendrograms (SPSS 1997) (Figures 5.6-5.8).

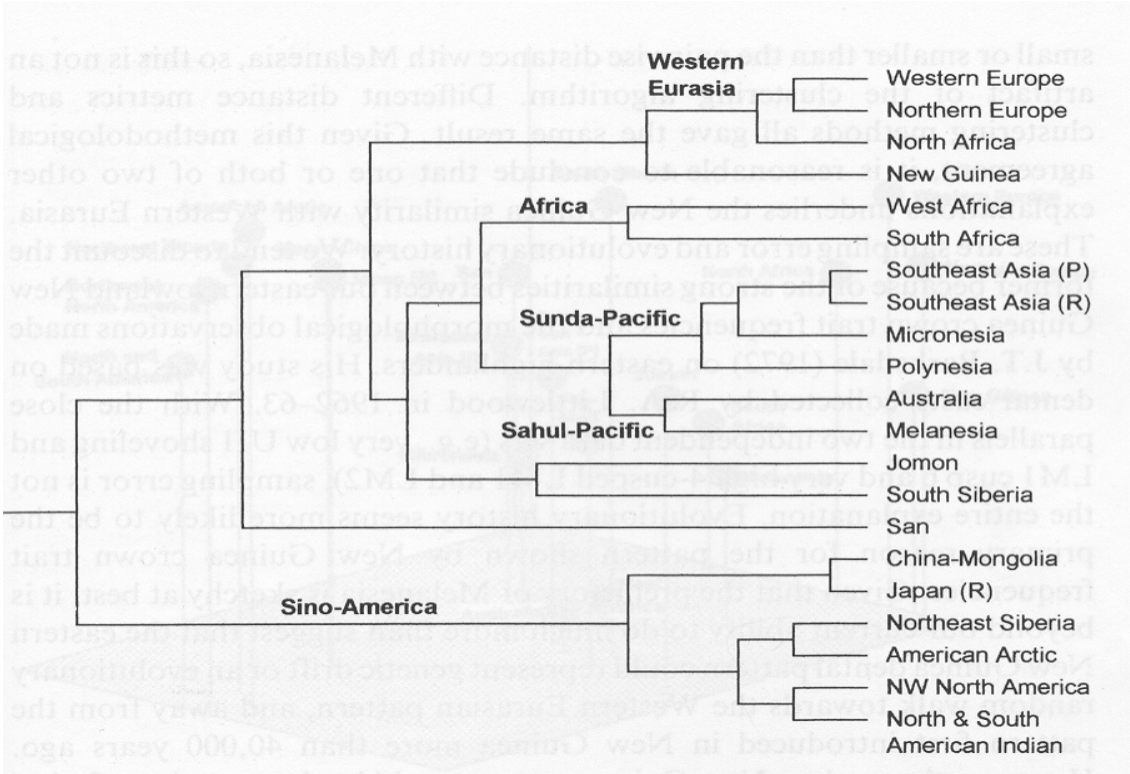

Figure 5.5: Worldwide populations based on 23 crown and root traits (Scott and Turner 1997)

Table 5.5: New York African Burial Ground Dental Traits Distribution

Dental Trait	Observations/ Total Sample
Winging/left	2/117
Shoveling ULI1	6/117
Double Shoveling ULI1	5/140
Interruption Groove LI2	15/136
Canine Mesial Ridge Left (Bushman)	19/122
Odontomes	1/117
3-cusped UM2	8/106
Carabelli's Cusp ULM1	18/123
Cusp 5 LUM1	24/120
Enamel Extensions ULM1	6/108
4-Cusped LM1	4/92
4-Cusped LM2	51/87
Y-Groove Pattern LLM2	26/95
Cusp 6 LLM1	7/95
Cusp 7 LLM1	11/102
Deflecting Wrinkle Lt	6/94
2-Rooted LUP1	42/76
3 Rooted LUM2	70/73
2-Rooted LC	0
Tome's Root Lt	32/84
3-Rooted LM1	0
1-Rooted LM2	1/63
Distal Trigonid Crest Lt	2/80

Based on Euclidean distance, the NYABG data are clustering closely to Scott and Turner's South African population data. Both, in turn, group closely to a second cluster involving Western Europe, Northern Europe, North Africa, and New Guinea. Interestingly, West Africa and the San population cluster together and distant from the other African populations (see Figure 5.6).

When the same distance analysis compares only the African populations and Western Europe, the NYABG clusters closely to North and South Africa, and then Western Europe. The West African and Khoisan populations fall farther from the previous groups (Figure 5.7). Lastly, when compared to only the African populations, the NYABG clusters closely to North Africa, followed closely by South Africa, and clustering farther from Western Africa (see Figure 5.8).

Scott and Turner's classification of North, South, and West Africa can, however, be misleading. The category labeled South Africa in this data set is made up of populations from South, East, Central, and West Africa (Table 5.6). Within this framework, the NYABG sample is actually clustering closely to a pooled sample of Africans south of the Sahara and some populations north of it.

Table 5.6: Scott and Turner Population Descriptions

WESTERN EURASIA:

Western Europe

Lapps, Reindeer Is., Karil Peninsula, England (Poundbury), Netherlands (Dorestad de Heul), Lent, Danish Neolithi

Northern Europe

Medieval Norway, Greenland, Iceland

North Africa

Algeria, Bedoin, Canary Islands, Carthage, Chad, Christian (Sudan), El Hesa, Kabyle, Kharga, Lisht, Meroitic, Mesolithic Nubians, Soleb, X-Group, North Africa (Algeria, Chad)

SUB-SAHARAN AFRICA

West Africa

West Africa, Nubia #117, Nubia #67/80

South Africa

Congo, Gabon, Ghana, Nigeria/Cameroon, Pygmy, South Africa, SeneGambia, Sotho, Tanzania, Togo/Benin, Tukulor

San

San, Khoi khoi

(1997: 318)

From the above analysis we can determine that the individuals from the NYABG sample are most biologically similar to individuals in West, Central, North, and South Africa. This trend continues as individual frequencies for each dental trait are partitioned. The **farthest** population clusters – thus the least biologically related to the NYABG – are from Northwest North America, North and South American, the American Artic (*ergo* Native Americans) China-Mongolia, Recent Japan and North East Siberia (Figure 5.6). As noted in our earlier discussion of craniometry, lumping diverse populations into arbitrary categories limits our ability to examine greater ethnic specificity with these methods. These data are, nonetheless, generally consistent with

regional origins of New York Africans reported by the African Burial Ground Project's historians.

Another problem of comparative databases might be resolved in future studies. As previously discussed most dental traits are measured in terms of grades, with the realization that scoring different levels of a grade is just as, if not more, relevant as simply noting its presence or absence. Even so, Scott and Turner (1997), when comparing world frequencies, analyzed a trait as either present or absent, or only considered the most prominent form of a morphological trait. For comparative purposes, we chose the same twenty-three dental traits and followed the same methodology. For example, Carabelli's Cusp is a dental trait found on the mesiolingual cusp of a maxillary molar. Carabelli's cusp can be exhibited in different grades ranging from a groove or pit to a free standing cusp in the same location. Following Scott and Turner's methodology, only grades 5-7 were considered in trait frequencies for comparison to other world populations. Here only 7 percent of the individuals from the NYABG (with observable dentition) exhibit this trait. However, if the lower grades (1-4) are considered unto themselves, then, 33 percent of the individuals exhibit Carabelli's. When all scores are considered (present or absent) 40 percent of the NYABG individuals exhibit some form of Carabelli's Trait. An increase in world-wide sample sizes has led to a more comprehensive understanding of how to interpret patterns of trait expression, through grades, rather than simply as present or absent.

Multivariate cluster analysis on the same twenty-three dental traits was used to determine genetic affinity within the NYABG sample. While small clusters are apparent, no major groupings are visible. However, a variation in methodology to include all

gradients of dental morphology may clarify this point. These data are consistent with the historical expectation of the NYABG sample as highly diverse, even if it consists of the expected range of African populations.

> *Tooth morphology is part of the biological heritage that humans carry with them when they migrate, much like their blood group genes, fingerprint patterns, PTC taste reactions, and other biological traits. When human groups are isolated from one another for a period of time, their crown and root trait frequencies diverge to varying degrees, depending on population size and the extent and temporal duration of isolation. When divergent populations come in contact and interbreed, the resulting populations possess convergent morphological trait frequencies. In other words, these polymorphic features of the dentition behave like other biological variables that are used to assess population history and evolutionary process.*
> *Scott and Turner, 1997*

Following Turner's theoretical perspective, the analysis of discrete dental traits strongly indicates that the NYABG sample is biologically similar. Multivariate analysis indicates a close degree of relationship between the NYABG and other African populations, particularly, West, Central and South Africa. Of note, is the consistent clustering of the NYABG with Scott and Turner's Western European population, which includes a sample from the Netherlands. There is a degree of relationship that is worth further investigation given the population history of New York City. These data add to an expanding database on world population dentition and demography. The use of dental morphology in the discussion of population movement is not unique. However, the NYABG provides an opportunity for investigating such techniques to shed light on a population of widely displaced individuals. That situation and the fact that teeth are visible in the living and the dead offer an opportunity to assess biological relationships between living populations, their relatives, ancestors and descendants. The NYABG as we have approached it also allows corroboration of these results against other biological

traits, and historical, cultural and artifactual evidence. Indeed, we have been guided by the preliminary archaeological data showing evidence of African cultural continuity in some funerary decorations at the site.

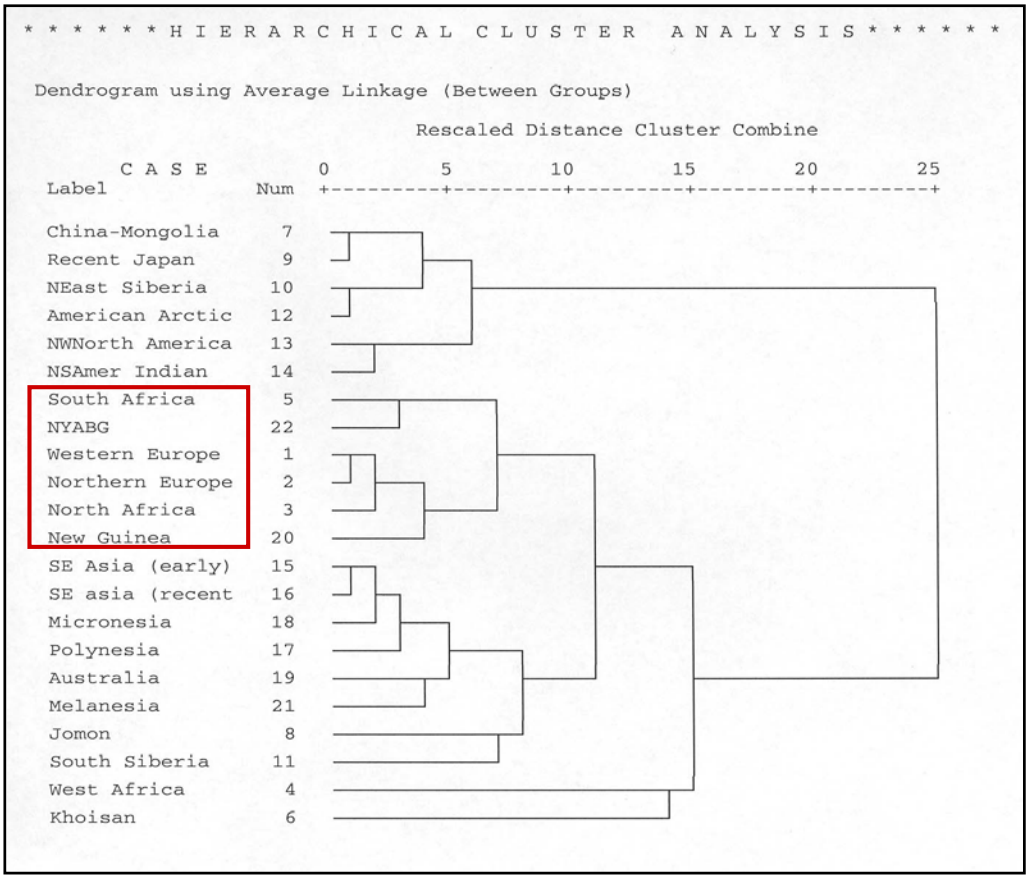

Figure 5.6: New York African Burial Ground compared to other world populations based on 23 crown and root traits (Scott and Turner 1997)

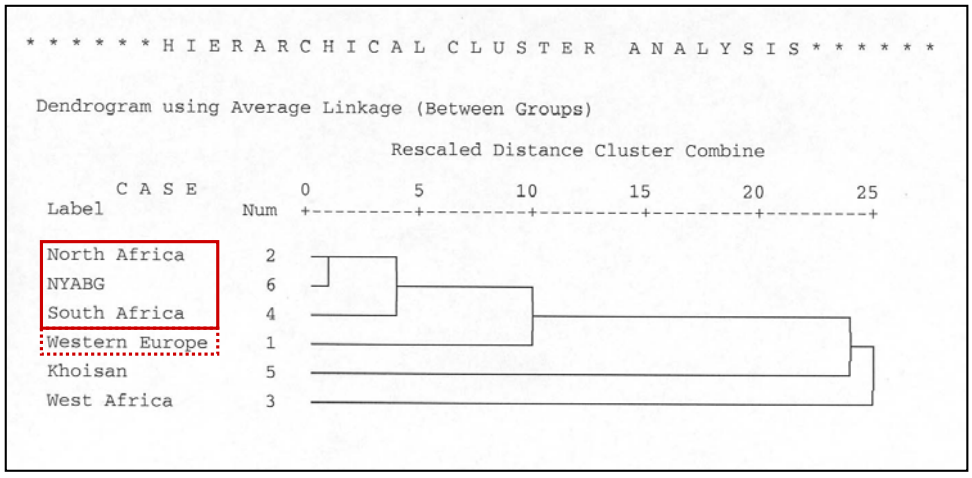

Figure 5.7: New York African Burial Ground compared to other African populations and Western Europe based on 23 crown and root traits (Scott and Turner 1997)

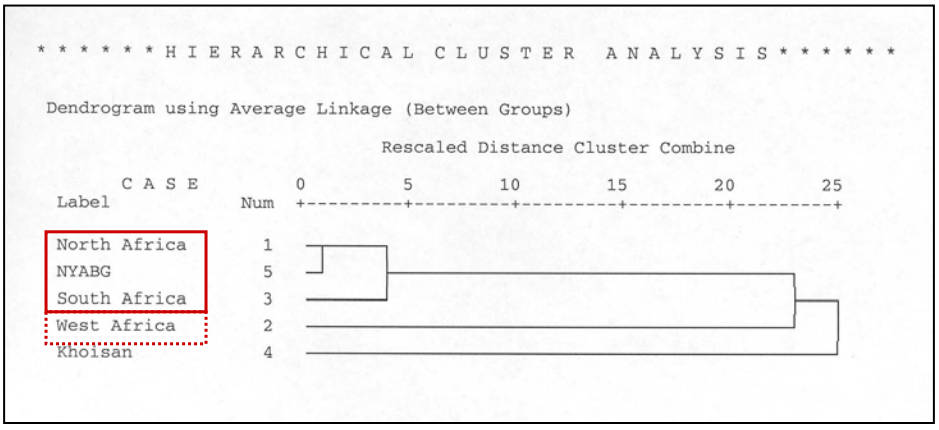

Figure 5.8: New York African Burial Ground compared to other African populations based on 23 crown and root traits (Scott and Turner 1997)

Molecular Genetic Assessments

Introduction: Overview, Limitations, and Approach

The genetic analyses of the NYABG samples provide an unparalleled opportunity for understanding the population origins and demographic structure of this unique group. Unfortunately, DNA extracted from these samples suffers from a fragmented genome and presence of PCR inhibitors, some of which were co-mingled with the extracted DNA. Critical to our analyses has been the quality of the aDNA. The quantity and quality of aDNA is dependent upon the interment conditions as well as the excavation specifics at the archaeological site. Therefore, we used the best standardized and established methods for aDNA analysis available at the time of analysis 1995-1999, so as to maximize genomic yield.

In 1995, GSA funded a small feasibility study with the following aims and objectives:

a. To isolate nucleic acids from bones and/or hair samples
b. Amplify specific mtDNA sequences via the PCR technique
c. Sequence the amplified products
d. Clone the amplified sequences for further study and provide a reservoir of these fragile sequences, and
e. Perform a phylogenetic analysis of the sequences to determine possible kinships and sites of origins for a small number of these individuals.

Initial work on this small feasibility study was lead by George and this feasibility study was then extended by Kittles. The details of our methods and materials are reported below.

1995-1997 Protocol for genetic analyses of the New York African Burial Ground

The initial subsample was identified in 1995. We were able to extract and isolate nucleic acids from nine 200-year old hair and bone samples by mid-June 1995. NYABG individuals at burial numbers 233, 274, 619, and 840 were amplified with primers L15,997 and H16,321. Burial Ground individuals 219, 267, 797, 253, and 274 were not amplified. Ancient DNA (aDNA) from the NYABG sample was done using the following standard methods:

Contamination control was maintained by providing separate rooms for extraction, amplification, and sequencing. All glassware, solutions, chemicals, instruments, and bones were rendered sterile either by autoclaving or by UV irradiation. The bones were further subjected to filing of a 1-2 mm layer of outer material to reduce the risk of surface contamination.

Pre-extraction processing techniques included breaking the cleaned and resurfaced bone into small pieces, wrapping it in previously autoclaved heavy duty aluminum foil, placing the wrapped bone in liquid nitrogen, and placing this between sterilized metal plates wrapped in heavy duty aluminum foil. The treated bone was then pounded into a fine powder using the metal plate. Liquid nitrogen was not required for the pre-extraction processing of the hair root samples.

DNA extraction and isolation involved subjecting 0.25 g of powdered bone to the silica/guanidinium thiocyanate extraction protocol of Bloom and colleagues (1990) and Hoss and Paabo (1993). In this protocol (discussed in more detail below), the

strategy was to release nucleic acids by enzyme digestion, bind these to a silica column, and later elute them for quantification.

PCR amplification was prepared for 5 μL of the 65 μL of nucleic acid extraction volume. Using specific oligonucleotide primer pairs, in which one of the pair had a biotinylated 5' end, the DNA is subjected to 35 cycles of PCR amplification (Hoss and Paabo 1993). The results were examined on a 2 percent NuSieve agarose gel.

Results

Results of the initial molecular genetic results were partially successful. aDNA was obtained from 9 of the 15 NYABG samples studied. Four of these nine samples were successfully amplified using specific mtDNA primers. The amplified mtDNA sequences from the skeletal remains were not successfully cloned using the TA cloning vector and the sequences were not subjected to phylogenetic analysis.

1998-1999 Protocols for Genetic Analyses of the New York African Burial Ground

The second subsample consisted of seven NYABG bone samples, some of which had been initially studied by George. Kittles was able to bring an updated methodology to the project. These studies included the following individuals identified by burial numbers: 219, 233, 274, 310, 383, 619, and 843.

Contamination prevention was maintained by extensive autoclaving (of all buffers and water), regularized filtration purification (of these buffers and water), exclusive use of disposable lab coats, gloves, sleeves, masks, and caps, and bleach wipes of equipment followed by UV light irradiation. All pipette tips contained filters, and all PCR reagents were separated into aliquots to reduce the risk of cross-contamination. The

mortars and pestles used to grind the bone to a fine dust were treated with 1N HCl, rinsed with double-distilled water, and subjected to UV radiation before each use.

Pre-treatment of bone for DNA extraction and isolation consisted of cutting off about 2 mm of the entire bone surface with scalpel blades. The resulting internal bone fragments (300-500 mg) were ground in a specimen dedicated mortar and pestle to a fine dust.

DNA extraction and isolation continued using a silica-based protocol. In this procedure, silica power was introduced to the digested sample, DNA bound under the influence of guanidinium thiocyanate, and this allowed the remainder of the contents of the digest to be washed away. In following this established technique, the ground bone was incubated in 1000 μl of guanidinium thiocyanate (GuSCN) extraction buffer overnight at room temperature resulting in the release of nucleic acids by enzyme digestion. The extraction buffer consisted of 4.7M GuSCN, 20 mM EDTA, 46 mM Tris (pH 8.0), and 1.2 percent Triton X-100. After incubating the solid tissue remains in the buffer, a pellet was produced by several centrifugations. The supernatants were then added to a silica suspension and nucleic acids were isolated, eluted in 30 μl aliquots, and quantified. As our experimental control, a blank extraction containing all reagents, but no tissue, was included in every set of extractions.

PCR amplification and sequencing was based on the amplification of the hypervariable segment I (HVS-1) of mtDNA and Y-chromosomal and autosomal microsatellite amplifications. Four sets of primers produced overlapping fragments of a 300 base pair segment of the HVS-1. Primers were also included which amplified highly

variable microsatellite markers of the Y-chromosome specific locus (DYS390) and the autosomal DNA locus (D5S471).

Amplification of mtDNA was performed in 30 μL reaction volumes of 150 μM dNTP's 10 mM Tris-HCl (pH=8.3), 50 mM KCl, 1.0-2.0 mM $MgCl_2$, 0.6 units of AmpliTaq polymerase, 3.0 μl of 5 μM primer mix and 7.5 μl of the DNA extract. The PCR conditions consisted of 40 cycles at 95°C for 50 seconds, 55°C for 50 seconds, and 72°C for 50 seconds. Amplification products were visualized on 3 percent agarose gels. Both DNA strands were then sequenced using fluorescent-labeled dideoxy terminator cycle sequencing chemistry using the ABI 373A DNA sequencer (ABI, Foster City, CA). The *Seq A* and *AutoAssembler* programs (ABI, Foster City, CA) were used to align and overlap both sequenced strands of DNA, allowing for the visual inspection of any ambiguities in the sequence.

Amplification of the Y-chromosome and autosomal microsatellites used 10 μL of DNA added to 200 μM of dNTPs, 10 mM Tris-HCl (pH=8.3), 50 mM KCl, 1.0-2.0 mM $MgCl_2$, 0.6 units of AmpliTaq polymerase (Perkin Elmer), and 0.33 μM of primers. The PCR cycling conditions were 93°C for 3 minutes, 10 cycles at 94°C for 15 sec, 55°C for 15 sec, and 72°C for 30 sec. Then samples were run at 20 cycles at 89°C for 15 sec, 55°C for 15 sec, and 72°C for 30 sec. The final extension cycle was at 72°C for 10 minutes.

Results

Results of the molecular genetic analyses of the second subsample indicated a strong West and/or Central African ancestral presence in the studied NYABG individuals. Only three of the mtDNA from sampled individuals exhibited unknown

molecular variants of mtDNA. Even in these cases, an African maternal ancestral origin may be present, as the background database on African mtDNA diversity is still in an early stage of development.

Analysis of the third subsample included 48 bone and two hair and/or tissue samples from the NYABG. Analyses were completed in 1999.

Contamination prevention was maintained by autoclaving and purification by filtration of all buffers and water. Disposable lab coats and gloves were used during all steps. Benches and equipment were treated with bleach and irradiated by UV light. All pipette tips contained filters. PCR reagents were separated into aliquots. The mortar and pestle (used to grind the bone into a fine dust) was treated with 1N HCl, rinsed and double-distilled (dd) water, and UV irradiated before each use.

Pre-extraction practices involved small samples of bone being cleaned by cutting off about 2 mm of the entire bone surface with sterile scalpel blades.

DNA extraction and isolation used internal bone fragments (300-500 mg) ground into a fine dust using a project-dedicated mortar and pestle. The ground bone was then incubated in 1000 μl of guanidinium thiocyanate (GuSCN) extraction buffer overnight at room temperature. The extraction buffer consisted of 4.7M GuSCN, 20 mM EDTA, 46 mM Tris (pH=8.0), and 1.2 percent Triton X-100. After incubation, the solid tissue remains were pelleted by centrifugation and supernatants added to a silica suspension. Nucleic acids were isolated and eluted into 30 μL aliquots. A blank extraction containing all reagents but no tissue was included in every set of extractions, as a control.

PCR amplification and sequencing used four sets of primers to amplify the hypervariable segment 1 (HVS-1) of mtDNA. The primers produced overlapping fragments of a 300 base pair segment of the HVS-1. Amplification of mtDNA was performed in 30 μL reaction volumes of 150 μM dNTP's 10 mM tris-HCl (pH=8.3), 50 mM KCl, 1.0-2.0 mM $MgCl_2$, 0.6 units of AmpliTaq polymerase, 3.0 μL of 5 μM primer mix and 7.5 μL of the DNA extract. The PCR conditions consisted of 40 cycles at 95°C for 50 sec, and 72°C for 50 sec. Amplification products were visualized on 3 percent agarose gels. Both DNA strands were then sequenced using fluorescent labeled dideosy terminator cycle sequencing chemistry (ABI) and the ABI 373A DNA sequencer (Foster City, CA). The *Seq A* and *AutoAssembler* programs (ABI, Foster City, CA). The *Seq A* and sequenced strands of DNA allowed for the visual inspection of ambiguities in the sequence. Sequence comparisons were accomplished using *PAUP Version 4.0* (Swofford 1999). Due to the large data set, an exact search was unfeasible, so extensive branch swapping was performed in order to find optimum trees.

Results

Results from the molecular genetic analysis of the third subsample allowed the comparison of our results with a database of published mtDNA sequences from around the world. Currently 1,800 sequences have been entered in the database. Accessible Native American and European sequences were represented among the published mtDNA sequences; however, of the 48 mtDNAs sequenced, 45 evidenced mtDNA haplogroups found in West and Central African populations and their recent descendants. The remaining three sequences were unknown, as previously noted. In the published mtDNA database, the number specific to African populations is about 849. Among these

849, those observed in West and Central Africa includes a total of 520 populations. The database includes individuals sampled from the following countries: Benin, Burkina Faso, Cameroon, Central African Republic, Guinea, Mali, Morocco, Niger, Nigeria, Senegal and Sierra Leone. For each country, we noted the geographical region and nearest probable historical export site for enslaved Africans bound for the Americas during the seventeenth and eighteenth centuries (Table 5.7):

Table 5.7: Countries, Geographical Regions, and Historical Export Sites for Enslaved Africans

MODERN COUNTRY Represented in the 1999 Database	GEOGRAPHICAL REGION Represented in the 1999 Database	NEAREST PROBABLE HISTORICAL SLAVE EXPORT SITE(S)
Benin	West Africa	Bight of Benin
Burkina Faso	West Africa	Gold Coast
Cameroon	West Central Africa	Bight of Biafra
Central African Republic	Central Africa	Bight of Biafra
Guinea	West Africa	Senegambia/Upper Guinea
Mali	West Africa	Senegambia
Morocco	Northwest Africa	Moroccan west coast
Niger	West Africa	Senegambia
Nigeria	West Africa	Bight of Biafra/Bight of Benin/Calabar
Senegal	West Africa	Senegambia
Sierra Leone	West Africa	Windward Coast

At the time of our analyses, no samples were included on the published database from Ghana, Angola, Gabon, Congo, Liberia, or other areas known historically to have included important catchment areas or export sites for the transatlantic trade in enslaved Africans to the Americas.

Nucleic acids were extracted from all 48 of the bone samples provided in this subset of the NYABG. Extractions from the two tissue samples, burials numbered 23 and

97, failed to yield adequate DNA. For the successful 48 DNA extractions, mtDNA control region sequences (<300 bp) were amplified by PCR and the products visualized using ethidium bromide stained agarose gels. Direct sequencing of the products revealed several polymorphic sites among the samples.

The level of genetic diversity observed in subsample three from the NYABG was quite high. Forty-five of the 48 sequences were unique and the haplotype diversity closely approached 1.0 (0.997 + 0.01). This high level of haplotype diversity is common for populations of African descent (Watson et al 1997; Vigilant et al. 1991). Countries, geographical regions, and macroethnic groups are listed when haplotypes appear restricted to such units. Sequences that are phylogenetically related to West or Central African sequences, but are not observed in any particular geographical region or among a specific macroethnic group are designated West/Central AFRICAN. Haplogroups are also noted. Although there has been limited and sporadic sampling of Africans for genetic studies, by 1999 many studies had identified at least three mtDNA haplogroups in African populations: L1, L2, and L3. Table 5.7 details the genetic affinity of samples as they relate phylogenetically to the published data (as of 1999).

All three haplogroups were observed in the third subsample from the NYABG individuals. Not surprisingly, the L1 haplogroup is observed in the least sampled geographical area of Africa; so we expect that it may be more common than reported. Haplogroup L2 is common among the Niger-Kordofanian speakers from the Senegambia and Gold Coast regions of West Africa. The L2 haplotypes, which may represent the descendants of migrants of Bantu speakers into West Africa, constitute 69.5 percent of the studied ABG individuals.

The third mtDNA haplogroup, L3, is quite common in East Africa and in the Horn region of Africa. While the L3 group is more common in East Africa, it is observed at an appreciable frequency in West Africa, particularly among Afro-Asiatic speakers. Since many of the enslaved Africans were derived from more inland areas of West and Central Africa, such as northern Nigeria, northern Cameroon, and southern Niger, for example, this may explain our observation of the L3 haplogroup in 21.7 percent of the NYABG individuals. Table 5.8 summarizes the molecular genetic affinities of the NYABG sample.

Table 5.8: Molecular genetic affinities of 48 individuals of the NYABG

Burial #	Catalog #	Tissue Site Sampled	mtDNA Haplo-group	Geographical, Country, And Macroethnic Genetic Affinity
1	93	R. Radius	L2	West/Central AFRICAN
6			L2	West Africa, Benin (Fulbe peoples)
7	Not Indicated	Not Indicated	L3	West Africa, Niger
9	233	R. Radius	L2	West Africa, Benin (Fulbe peoples)
11	267	R. Ulna	L2	West/Central AFRICAN
12	Not Indicated	Not Indicated	L2	West/Central AFRICAN
16	326	R. Ulna	L2	West/Central AFRICAN
20	310	R. Fibula	L2	West/Central AFRICAN
25	358	R. Ulna	L3	West/Central AFRICAN
32			L3	West Africa, Niger
37	460	R. Fibula	L2	West/Central AFRICAN
40	489	R. Fibula	L3	West Africa, Niger
47	619	R. Ulna	L2	West Africa, Benin (Fulbe peoples)
49	641	R. Fibula	L2	West/Central AFRICAN
51	700	R. Fibula	L2	West/Central AFRICAN
56	793	R. Radius	L3	West Africa, Niger
58	Not Indicated	Not Indicated	L2	West/Central AFRICAN
63	Not Indicated	Not Indicated	L2	West/Central AFRICAN
67	810	R. Radius	L2	West/Central AFRICAN
71	813		L2	West/Central AFRICAN
73	815	R. Radius	L2	West Africa, Nigeria (Yoruba peoples)
76	819	R. Fibula	L3	West Africa, Niger
89	830	R. Ulna	L1	West/Central AFRICAN
97	840	R. Ulna	L2	West Africa, Nigeria (Fulbe peoples)
101	843	Not Indicated	L3	West Africa, Niger
105	Not Indicated	Not Indicated	L1	West/Central AFRICAN
107	850	R. Fibula	L2	West Africa, Nigeria (Hausa peoples)
115	858	R. Fibula	L3	West Africa, Niger
122	867	R. Ulna	L2	West Africa, Nigeria (Hausa peoples)
135	878	R. Fibula	L2	West//Central AFRICAN
138	883	R. Fibula	L2	West Africa, Senegal (Mandinka peoples)
144	Not Indicated	Not Indicated		West/Central AFRICAN
151	896	R. Ulna	L2	West/Central AFRICAN
154	899	R. Fibula	L3	West Africa, Niger
158	903	R. Fibula	L2	West Africa, Senegal (Mandinka peoples)
171	931	R. Ulna	L1	West/Central AFRICAN
176	Not Indicated	Not Indicated	L2	West/Central AFRICAN
180	960	R. Radius	L2	West Africa, Senegal (Mandinka peoples)
194	Not Indicated	Not Indicated	L2	West Africa, Nigeria (Fulbe peoples)
219		R. Fibula	L3	West Africa, Niger
226	Not Indicated	Not Indicated	L2	West/Central AFRICAN
233	Not Indicated	Not Indicated	L2	West Africa, Benin (Fulbe peoples)
242		R. Fibula	L2	West Africa, Nigeria (Fulbe peoples)
310		R. Rib	L2	West/Central AFRICAN
335		R. Ulna	L2	West/Central AFRICAN
340	Not Indicated	Not Indicated	L2	West Africa, Nigeria (Fulbe peoples)

Genetic Initiatives and Protocols for 2000 – 2004

When 219 NYABG samples were transferred to the Bioanthropology Research Laboratory at the University of Maryland in 2000, our initial assessment identified four major problem areas:

1. Inadequate database on contemporary and archaic African genetic
2. Diversity High levels of intra-African genetic variability
3. Complex ethnic histories and demographic patterns
4. Difficulty in extracting sufficient quantities (and quality) of archaic skeletal DNA for multiple analyses

Our solutions were to:

1. Establish an International Advisory Board of senior anthropological geneticists
2. Identify historians and anthropologists with specific regional expertise
3. Utilize pooled regional samples to recreate regional clusters of marker genes (possibly using DNA microarrays)
4. Apply advanced biotechnological techniques to recover ancient DNA and test against regional pools
5. Set up a National African DNA Bank(s) for future reference

Our initial focus was to address the serious lapses in the exiting database on African genetic diversity on the continent and throughout the Atlantic diaspora. For some time we had known that the limitations in the existing comparative database posed a significant hindrance to the reliable placement of NYABG individuals in particular geographical regions of Africa and among specific contemporary African macroethnic

groups. In an effort to begin to tie particular genetic variants to specific regional areas of the world, we began several important initiatives.

In 2000, several senior geneticists agreed to serve on an advisory board related to genetic analyses for the NYABG. They were Dr. Kenneth Kidd (Yale University), Dr. Kenneth Weiss (Pennsylvania State University), Dr. Michael Crawford (University of Kansas), Dr. Robert Ferrell (University of Pittsburgh), Dr. Alain Froment (Orleans University) and Dr. Robert Murray (Howard University).

Information from the ABGP historians suggested that West Central Africa was an important source of the Africans of Eighteenth Century New York. Therefore, we recruited and worked closely with the following regional experts:

- Dr. Paul Nkwi, Executive Secretary, Cameroon Academy of Sciences, Editor of *African Anthropologist,* and internationally known social anthropologist
- Professor Victor Ngu, President, Cameroon Academy of Sciences, noted physician and inventor
- Dr. Peter Ndumbe, Dean, Medical School, University of Yaounde 1, physician, specialist in infectious disease, and director of the Research Institute associated with the Medical School
- Dr. Ugo Nwokeji, Professor of History, University of Connecticut, expert on the export of West and Central Africans during the transatlantic slave trade
- Dr. Charles Dimintyeye, Cultural Attaché, Embassy of the Republic of Cameroon, Washington, DC, professor of French and expert on francophone West and Central Africa

- Professor Joseph-Marie Essomba, Professor of Archaeology, University of Yaounde I, expert on archaic evidence for human occupation in West and Central Africa.

In 2000-2002 efforts were underway to develop the first human DNA bank in Africa. Dr. Fatimah Jackson made two critical collaborative contacts: Dr. Jeanne Beck, Vice President of Coriell Institute for Medical Research, Camden, NJ and Dr. Peter Ndumbe, Dean of the Medical School, University of Yaounde I, Yaounde, Cameroon. After a series of meetings and working sessions with scientists at Coriell Institute for Medical Research (New Jersey Medical and Dental School) and the University of Yaounde I College of Medicine (Yaounde, Cameroon, West Central Africa), plans were implemented to lay the foundations for this bank. Cameroon's central location in Africa, highly genetically diverse population, as well as the presence of an adequately developed infrastructure and enthusiastic and supportive scientific and political communities made the country an ideal choice for housing this bank. Coriell Institute for Medical Research offered to train Cameroonian technicians in DNA banking techniques. The University of Yaounde I offered its Research Institute as a permanent site for the bank. With the permission and support of the Cameroonian Academy of Sciences, the Cameroon Prime Minister's Office, and the Ministry of Health, the bank was formally begun in July 2002. In November 2002 an international workshop was held in Yaounde, Cameroon where the goals and objectives of the bank were outlined, its direct relationship to the ABGP indicated (Dr. Michael Blakey was among the participants at the workshop), and the plans were laid for a collaborative grant proposal to the National Institutes of Health to support the bank. To date, the bank has already collected and extracted DNA samples

from over 400 West and Central Africans and is officially linked, through the Ministry of Tourism, with UNESCO's "Route of the Slaves" Project.

In 2002, Dr. Jackson began discussions with technical experts at Affimetryx Corporation to develop a DNA microarray that would provide rapid assessments of African regional markers. At that time, each gene chip could contain 2,000 single nucleotide polymorphisms (SNPs). Our plan is to identify, through the literature and through direct collections, regional African variation in SNPs. SNP variation among specific African regions will then be used to design custom-made DNA microarrays for target testing, analysis (bioinformatics), and interpretation. The geographical regions identified as major sources of genetic polymorphism for Eighteenth Century New York Africans include: Central Africa, Bight of Biafra, Mozambique, Senegambia, Upper Guinea, Bight of Benin, and the Gold Coast. We continue to concentrate our research on groups from these geographical regions as they provide the strongest baseline information on the NYABG ancestral template; these regions provide insights into Eighteenth Century levels of African-American genetic sequence polymorphisms. Further, these regions most powerfully permit reconstructions of African and non-African origins. Our methods for these ethnogenetic reconstructions include the following:

1. Archaic Map Analysis
2. Regional Ethnic Reconstructions
3. Group Displacement Tracking
4. Ethnic and Regional Verification using Alternative Documentation
5. Geographical Information System (GIS) Mapping using Vector and Raster Maps

6. Contemporization of the findings (i.e., determining the modern equivalents)
7. Statistical Analysis

In 2003-2004 Dr. Jackson received Institution Review Board (IRB) clearance to initiate genetic studies among the African-descended student, faculty, and staff population at the University of Maryland. The aims of this project were to:

1. Attract 100 to 200 African, Afro-Caribbean, and African-American students currently enrolled at the University of Maryland to a workshop-dinner on genetics and health;
2. Provide these students with an opportunity to extract DNA from various fruits, to learn about some of the latest advances in the genetics of disorders disproportionately affecting peoples of African descent; and
3. Collect and analyze buccal cell samples from each student for the presence of genes associated with specific regional origins. This DNA will become part of a database for future comparative analyses with the NYABG individuals.

To date, we have collected 183 DNA samples from individuals from all over the world, including Europe (Scotland, Ireland, Denmark, Germany, Italy, Spain), South America (Brazil, Colombia, Peru), Asia (Nepal, India, Butan, Pakistan, China, Korea) and Africa (Ghana, Nigeria, Cameroon, Liberia, Congo, Guinea, Ethiopia, Senegal, Kenya, Tanzania), as well as the United States. In April 2004, our first paper was presented at the annual meetings of the American Association of Physical Anthropology entitled "African-American lineage markers: determining the geographic source of mtDNA and Y chromosomes" (Lorenz et al. 2004).

Future Genetic Studies of the New York African Burial Ground Samples
Specific Genomic Segments to be Evaluated

Continuation of the mtDNA Studies

Over the last 9 years, our research team has clearly demonstrated the ability to successfully extract, isolate, sequence, and analyze mtDNA from 200-300 year old skeletal material. Using the improved techniques now available, our continued studies of MtDNA present an excellent opportunity to make within-group delineations, particularly among Africans. The mtDNA haplogroup **L3e**, which is identified by the restriction site +2349 MboI within the Afro-Eurasian superhaplogroup **L3** (-3592 HpaI), is omnipresent in Africa but virtually absent in Eurasia (except for neighboring areas with limited genetic exchange). **L3e** had previously been poorly characterized in terms of HVS-I motifs, as the ancestral HVS-I type of L3e cannot be distinguished from the putative HVS-I ancestor of the entire **L3** (differing from the CRS by a transition at np 16223). However, recently Bandelt and colleagues (2001) undertook the MboI screening at np 2349 of a large number of Brazilian and Caribbean mtDNAs (encompassing numerous mtDNAs of African ancestry), and revealed that **L3e** is subdivided into four principal clades, each characterized by a single mutation in HVS-I, with additional support coming from HVS-II and partial RFLP analysis. Apparently the oldest of these clades (transition at np 16327) occurs mainly in central Africa and was probably carried to southern Africa with the expansions of the Bantu peoples. The most frequent clade seems to be prominent in many Bantu groups from all of Africa. In contrast, a second clade is essentially restricted to Atlantic Western Africa (including Cabo Verde). This should permit us to distinguish between Senegambians and Central Africans within the NYABG sample. Our most recent work on mtDNA compares Cameroonians, Senegalese, and African

Americans and suggests significant differences in **L1/L2**, **L3d**, and **L3e** among the three groups: (see Table 5.9)

Table 5.9: Distribution of Haplogroups in the African and African-American Populations

Population	L1/L2	L3b	L3d	L3e	Non-L	Unknown
Cameroon	43.2%	7.4%	3.7%	19.8%	9.9%	16%
Senegal	61.1%	9.7%	6.2%	9.7%	10.6%	2.7%
African American	50.6%	7.1%	9.4%	14.1%	18.8%	0%

Of particular note are the 16 percent unknown (meaning samples that did not amplify for one or more haplogroup markers) among the Cameroonians (compared with 2.7 percent among the Senegalese and 0 percent among the African Americans) and the 18.8 percent non-L among the African Americans (probably an indicator of past maternal gene flow from non-African individuals).

Continuation of Y-Chromosome and Autosomal Studies

In addition to the mtDNA studies, we plan to return to our investigations of the Y-chromosome. The Y-chromosome DNA polymorphisms have been studied extensively and found to be useful in distinguishing between major continental groups as well as detecting within group variability. At least 13 years ago, Torroni and colleagues (1990) observed that several Y-specific TaqI fragments are recognized by 49a and 49f probes in human male DNA digests. The occurrence of polymorphic variations in six of these fragments (A, B, C, D, F and I) has already been well reported in the published literature, and these provide a potentially powerful tool for the study of the population genetics of the Y chromosome. The 49a-49f/TaqI polymorphisms were studied recently in 121

Africans from Senegal and Cameroon, two important origins sites for the Africans of the NYABG, and 125 Europeans from Italy. Four new bands were observed, of which, three (new) patterns were found. In each of these patterns, bands were consistently present in which (G, O and H-P-R) were missing. However, among the Africans tested, all lacked C and D fragments. Moreover, a band, A1, characterized about 80 percent of the Senegalese and Cameroonians, but was not present in the Italian group. The combination of A1C0D0 could therefore be a powerful genetic marker of paternal West or West Central African ancestry. This combination occurs in five haplotypes, one of which, haplotype IV, accounts for 68 percent of the African sample. In contrast with the results of the mtDNA analysis on the same population samples, the degree of variability displayed by these (and other) Y chromosome sequences appears to be much lower in Africans than in Europeans. For example, Watkins and colleagues (2001) analyzed 35 widely distributed, polymorphic Y-chromosome Alu loci in 715 individuals from 31 world populations. The average frequency of Alu insertions (the derived state) was lowest in Africa (.42) but higher and similar in India (.55), Europe (.56), and Asia (.57). A comparison with 30 restriction-site polymorphisms (RSPs) for which the ancestral state has been determined shows that the frequency of derived RSP alleles is also lower in Africa (.35) than it is in Asia (.45) and in Europe (.46). Neighbor-joining networks based on Alu insertions or RSPs are rooted in Africa and show African populations as separate from other populations, with high statistical support. Correlations between genetic distances based on Alu and nuclear RSPs, STR polymorphisms, and mtDNA, in the same individuals, were high and significant. Y-chromosome data are important in our biological affinity reconstructions in that we anticipate that we will encounter non-African Y-chromosome

haplogroups in some proportion of the NYABG individuals born outside of Africa (e.g. in the Americas) and interred during the Eighteenth century in particular.

Given the limitations of aDNA, we plan also to study the distributions in the NYABG of the following tetrameric short tandem repeat (STR) polymorphisms: STR loci D3S1358, VWA, FGA, D8S1179, D21S11, D18S51, D5S818, D13S317, D7S820, CSF1PO, TPOX, TH01, and D16S539.

Application of Specific Genomic Segments to Particular Research Questions

The three applied genetics topics that will be investigated will be ancestral origin, biological affinity, and molecular sex. We propose to test hypotheses that revolve around four central questions:

1. What geographical regions of Africa are represented in the NYABG individuals?
2. What degree of biological affinity exists among closely interred individuals in various sections of the NYABG?
3. What is the relationship between molecular sex and anthropometric sex in the ABG population?
4. Can we distinguish molecular sex-linked differences in various sources of stress (age, infection, nutrition, biomechanical) in the NYABG individuals?

mtDNA analysis is central to our ancestral origins and biological affinity analyses because of the anticipated low level of admixture in the earliest burials of the NYABG. Following these analyses in importance are our assessments of the Y-chromosome STRs. The importance of these data in biological affinity studies and molecular sex determinations is reflected in the initial sex ratio disparities in importation of enslaved

Africans into New York City's enslaved population base. Both mtDNA and Y-chromosome STR studies however must be supplemented with other autosomal genetic information (STRs) as discussed above. These nuclear DNA based data are essential for two major reasons. They fill in the biological lineage reconstructions by including the non-gender based ancestral genetic contributions, and they are necessary to begin to localize geographically the probable historical ancestral origins of studied individuals. Recent studies have found that fragments of up to 800 bp in length can be reproducibly amplified from aDNA extracts (Haack et al. 2000). In particular, we plan to amplify the short tandem repeat (STR) locus HUMVWA31A, and use this to address, in part, the biological affinity and grave location questions as well as address the development of individualized allelic profiles. Authentication of the amplified fragments will be carried out by measures of expectancy.

Ancestral Origin Studies: Research Hypotheses and Analytical Methods

From our previous studies of various subsamples of the NYABG, it is clear that it is possible to identify the ancestral origin of NYABG individuals and to determine, for an important proportion, their likely geographical regions within West and Central Africa (and even their probable macroethnic affiliations). These intriguing results revealed in 1999 suggest that even further refinements are possible using the expanded databases (public and private) to which we now have access. The geographical regions of West and Central Africa on which we are focusing our DNA microarray efforts are elaborated in Table 5.10.

Table 5.10: African Geographical Regions Currently Under Investigation as Sources of Genetic Polymorphisms

Geographical Region (based on historical slave export sites)	Inclusive Modern Countries	Status of Genetic Investigations (as of June 2004)
Central Africa	Angola, Republic of Congo, Democratic Republic of Congo, Gabon, Equatorial Guinea, Cameroon, Central African Republic, northern Namibia	Contact made with Embassies of Angola, Gabon, Cameroon. Establishment of National human DNA bank in Cameroon. Formal collaborative relationship with Cameroon Academy of Sciences, UNESCO "Route of the Slaves" project.
Bight of Biafra	Western Cameroon, Eastern Nigeria	DNA collections made in western Cameroon
Mozambique	Mozambique, Madagascar	Contact made with Embassy of Mozambique, official correspondence with colleagues in Madagascar
Senegambia	Senegal, Gambia, Northern Guinea, Southern Mali, Southern Mauritania, Guinea Bissau	DNA collections made in Senegal. Contact made with Embassy of Mauritania, correspondence with colleagues in Guinea
Upper Guinea	Southern and Western Guinea northwestern parts of the Windward Coast (Sierra Leone and Liberia)	DNA collections made in Liberia. Limited genetic collections from Sierra Leone
Bight of Benin	Benin, Western Nigeria, Southeast Niger	
Gold Coast	Ghana, Burkina Faso, Eastern Cotê d'Ivoire, Southeast Niger	DNA collections from Ghana
Other	Western Morocco	Contact with Embassy of Morocco

Basically, our earlier genetic studies of subsamples of the NYABG have begun to address both of the two major research questions we considered when we began our investigations in 1995:

1. Can we distinguish between continental groups (Africans, Europeans, and Native Americans as a subset of Asians) at the genetic (and/or phenotypic) level(s)? If this is possible, then the second issue is:

2. Can we distinguish genetically (and/or phenotypically) among Africans, among Europeans, and among Native Americans coming from various historically relevant areas and germane ethnic groups within a specific continent? Most importantly, since the individuals of the ABG appear to be Africans, can we distinguish among Africans from various geographical regions and between different localized macroethnic groups of Africa?

The continued ancestral origins analysis of the NYABG individuals is aimed at determining where in Africa they likely came from and, when possible, from among which local and regional groups. These data will make an important contribution to anthropological genetics, African diasporic history, US colonial history, and our understanding of the peopling of Eighteenth Century New York.

Biological Affinity Studies: Research Hypotheses and Analytical Methods

The burial locations of interred individuals and the biological lineage relationships among co-interred and closely interred individuals are the foci of our first cluster of null and alternative hypotheses. There are numerous shared graves in the NYABG of women with infants and older children buried with younger children. In burials 326 and 374 there is evidence of an adult male of about age 50 co-interred in the same grave shaft with an infant under six months of age. Molecular analyses should indicate to what extent these two individuals were biologically related. Another application of the molecular testing of inter-individual biological affinities addresses the interrelationships of closely interred individuals as well as the affinities of all 400 studied

NYABG individuals. Were the various sections of the excavated burial ground a final resting place for related individuals, for one gender versus both, for infants, children and subadults and not adults? Was the total excavated section of the NYABG a familial or ethnically delineated portion of the overall Burial Ground and thus housed family groups? Or, were the burial sites, in general, the final resting places of biologically unrelated individuals? In terms of kinship and biological lineage affinity, does the placement of deceased individuals in the NYABG have any significance?

For addressing these questions, we have developed the following cluster of specific null hypotheses:

- H_oI: Interred individuals of the NYABG show no evidence of overall or sectional structured burial placement by molecular sex identity and/or by age.
- H_oII: Co-interred individuals of the NYABG show no greater evidence of biological affinity as measured by molecular genetics than that seen among the NYABG residents in general.
- H_oIII: Geographically closely interred individuals of the NYABG show no greater evidence of biological affinity than that seen among the NYABG residents in general.
- H_oIV: Burial placement is uninfluenced by various indices of stress, including infection, nutritional deficiency, or biomechanical stress.

The alternative hypotheses regarding the intra-group biological affinity of the NYABG sample include:

- H_aI: Interred individuals of the NYABG show evidence of overall patterned interment by age, molecular sex, and familial groups including sectional sex groupings, sectional age groupings, and sectional familial groups (based on evidence of structured biological lineage affinities).
- H_aII: Co-interred individuals of the NYABG show greater biological affinity to each other than that seen in general among NYABG residents.
- H_aIII: Geographically closely interred individuals of the NYAGB show stronger biological affinity than do the individuals of more distantly placed burials.
- H_aIV: Among interred individuals, burial placement is influenced by osteological evidence of infectious disease but not by evidence of nutritional deficiency or biomechanical stress.

We plan to study the burial ground by initially partitioning it into sections based upon the designations of the NYABG archaeologists, noting the arrangements of coffins, existing barriers on site, and the timing and vertical placements of burials. Next, we will merge these sections to do comprehensive overall assessments. We will be looking for clusters of STRs, mtDNA, and Y-chromosome haplotypes that correspond to geographic proximity of burial site. We are particularly interested in the genetic analyses of the following co-interred burial dyads and triads:12 and 14; 25 and 32; 72, 83, and 84; 79 and 90; 89 and 107; 94 and 96; 121 and 202; 142, 144, and 149; 146 and 145; 159 and 161; 225 and 252; 226 and 221; 255 and 265; 263 and 272; 268 and 286; 219 and 235; 311 and 316; 314 and 338; 318 and 321; 320 and 334; and 326 and 374.

To test the hypotheses (I-IV) presented above, the molecular genetic profiles (aDNA-based) of all interred individuals will be studied and correlated with geographical proximity of interment site. Correlation coefficients and multi-way analysis of variance will be determined for extensive within-group comparisons, both in the archaeologically designated sections and for the overall burial ground area. An alpha value of 0.05 will be considered significant at both levels of assessment (within section and overall).

Due to the extensiveness of the NYABG area, we will use Geographical Information Systems software (GIS), discussed below, to plot the burial ground (using a raster maps) and then superimpose upon these raster maps the genetic and osteological information. The sections will be merged using GIS to produce general and specific maps; this should permit faster, better-integrated, 3-dimensional assessments of the interrelationship of geographical proximity and biological affinity. This approach is particularly important since biological affinity may be evident in some sections of the burial ground but not in other sections. Furthermore, the use of GIS will permit easily visualized evidence of any patterning or substructuring with respect to burial position and biological lineage affinity as well as its statistical assessment.

Application of Geographical Information Systems (GIS) to New York African Burial Ground

GIS will be used to effectively depict multi-tiered information on the NYABG by transforming geographic, historical, osteological, and molecular data into vector and raster maps using either AutoCAD (AutoDesk), ArcView (ESRI) or Arc/Info (ESRI). Vector maps will be produced for demarcated sections of the burial ground as well as for the burial ground as a whole. The locations of clustered coffins and multi-person burials

will be noted by defining each feature of the NYABG by a point in space. We will then connect the points to draw lines and area outlines. Image data will be added to our vector data to provide general geographical points of reference. The analysis of vector data involved summarizing the attributes in the layers of molecular sex, age, infectious disease, nutritional deficiency, biomechanical stress, and molecular genetic markers data tables. Raster maps will be used for continuous numeric values (such as intensity of infectious disease) using the age clusters as our cell size. ArcView's Spatial Analyst will allow us to sample, model, and grid raster based data (reclassification, interpolation, creation of surfaces).

Molecular Sex Determinations: Research Hypotheses and Analytical Methods

We plan to study the molecular sex of the NYABG individuals, focusing first on the unsexed infants, young children, and subadults among the interred. Dr. Jackson has taken the lead in writing a proposal to the National Science Foundation seeking support to identify the molecular sex of all individuals recovered from the NYABG and then comparing these results with their anthropometric sex, when available. With this unique database in place, we hope to test a number of hypotheses aimed at exploring gender bias in mortality by age, by osteological evidence of infection, nutritional deficiencies, and biomechanical stress, and by physical location within the section of the NYABG excavated. As mentioned in the previous section, we also seek to identify the molecular sex of co-interred individuals as well as individuals buried in close proximity within various sections of the excavated NYABG.

All molecular sex determinations will be based on standardized aDNA analyses while all anthropometric sex determinations, as well as evidence of osteological infections and biomechanical stress, will derive from recently completed assessments of the skeletal biology research team of the NYABG.

Since sex identity has an important influence on so many other variables, constructing the sex composition of the sample is an essential component in fully characterizing the NYABG individuals. Morphological and morphometrical analyses of skeletal remains usually give reliable access to the gender of mature individuals while the analyses of skeletal remains of immature individuals allocate only 70-90 percent of the individuals (Lassen et al 1997). In the NYABG, 65 infants, 44 children, 24 subadults, and X adults remain unsexed. For these individuals, molecular sex data will be invaluable.

Therefore, the second cluster of specific null hypotheses includes the following:

- H_oV: Molecular sex determinations correspond with (are identical to) anthropometric sex determinations.
- H_oVI: No molecular sex bias is evident in the pooled sample; overall, the number of genetic males equals the number of genetic females in the NYABG sample.
- H_oVII: No molecular sex bias is evident at any age group among infants, children, subadults, and adults in the sample.
- H_oIX: No molecular sex bias is evident by osteologically-evident infection in any age group among infants, children, subadults, or adults in the sample.

- H_oX: No molecular sex bias in evident by osteologically-evident nutritional deficiencies in any age group among infants, children, subadults, or adults in the sample.
- H_oXI No molecular sex bias is evident by biomechanical stress in any age group among children, subadults, or adults in the sample.

The second cluster of alternative hypotheses regarding molecular sex includes:

- H_aV: Molecular sex determinations do not correspond with anthropometric sex determinations; molecular sex determinations indicate more females than were evident anthropometrically.
- H_aVI: There is evidence of molecular sex bias among pooled interred individuals, and the number of genetic males is greater than the number of genetic females.
- H_aVII: There is evidence of molecular sex bias by age with more genetic males than genetic females at each age group among infants, children, subadults, and adults in the sample.
- H_aIX: There is evidence of molecular sex bias by osteologically-evident infection in each age group with genetic females showing more osteologically-evident infection than genetic males.
- H_aX: There is evidence of molecular sex bias by osteologically-evident nutritional deficiencies in each age group with genetic females showing more osteologically-evident nutritional deficiencies than genetic males.

- *H_aXI:* There is evidence of molecular sex bias by biomechanical stress in each age group with genetic males showing more biomechanical stress than genetic females.

To test the above-referenced null hypotheses (*V-XI*), we will use extracted aDNA from each interred individual to characterize and identify molecular sex from bone samples from the NYABG individuals, which have already been collected from different skeletal elements. Ancient DNA (aDNA) will be isolated from these skeletal elements by a combination of automated phenol/chloroform extraction and precipitation with silica powder. A combination of manual Chelex extraction and a purification kit will also be used to perform an extraction (as per Lassen et al. 2000). Finally, the aDNA extracts will be amplified with a primer system that amplifies a part of the amelogenin gene located on the human sex chromosomes. These characterizations will be performed double-blind; that is, we will not be aware of the previously done anthropometric sex determinations while the molecular sex identities are being determined. Further, the anthropometric sex determinations will have been made without knowledge of the molecular sex identities. These latter molecular sex identities will then be compared statistically to determine the correspondence between molecular genetic and anthropometric results. We will then return to the molecular sex identities and partition the interred individuals by their molecular sex and osteologically determined age groupings. Molecular sex determinations will be especially important for the interred NYABG infants and young children for whom it was often impossible to determine anthropometric sex. An alpha level of 0.05 will be considered statistically significant. This strategy will also allow us to effectively consider the alternative hypotheses associated with this first cluster.

Summary of Planned Future Analyses and Proposed Timetable

The genetic analysis of the NYABG provides a unique opportunity to explore and understand human biology and biodiversity at a very technologically sophisticated level. To our knowledge, our studies represent the first attempt to characterize an African/African-American historical population at the molecular genetic level of assessment. With full access to the NYABG samples and adequate time to complete these analyses, we feel that a major contribution can be made to the knowledge base, with positive effects for the entire nation. Table 5.11 summarizes the planned future analyses, the timetable for these analyses, and the support structures already in place to address these analyses.

Table 5.11: Anticipated Future Genetic Analyses of the NYABG Samples

Type of Analysis	Relevant Genomic Segments for study	Project Initiation Date	Anticipated Project Completion Date	Project Support Status as of June 2004
NYABG Ancestral Origins (African, European, Native American)	mtDNA haplogroups, Y-chromosome and autosomal STRs	2002	2009	Funding received from David C. Driskell Center for Diaspora Studies (UM) and Nyumburu Cultural Center (UM). Currently working on NIH proposal with colleagues at Coriell Institute for Medical Research
Biological Affinities among NYABG individuals	mtDNA haplogroups, Y-chromosome and autosomal STRs	2003	2007	Critical feedback received from NYABG archaeologists for "in progress" NSF grant proposal
Molecular Sex of NYABG individuals	Amelogenin gene located on the human sex chromosomes	2004	2008	NSF grant proposal in advanced stage of development